From oligonucleotide synthesis to peptide-PMO for treatment of neuromuscular diseases

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During my postdoctoral studies in 1973-75 with Gobind at MIT, I was involved in synthesis of oligodeoxyribonucleotides towards synthesis by classical phosphodiester chemistry of a Tyr suppressor tRNA gene. In the late 1970s at the MRC-LMB I worked on a new solid phase method of synthesis on a polyamide support. Initially we were able to extend a growing oligonucleotide chain by one nucleotide unit using diester chemistry in 2 days (1977-79), then using triester chemistry in 4 hours (1979-1982) and finally in 1 hour (1983-84), such that an oligonucleotide could be synthesized and purified within 2 days. Subsequently the machine-aided phosphoramidite synthetic methods of Marv Caruthers and others shortened the time further and reduced the labour needed considerably. Accordingly we moved on in the late 1980s to methods of end labeling for mRNA detection as well as synthesis of RNA and their analogues for applications in studies of ribozyme function. Also at this time we learnt DNA cloning and Sanger shotgun DNA sequencing resulting in the cloning, expression and mutagenesis of T4 RNA ligase, previously thought to be unclonable.

During the 1980s and 1990s several companies were developing antisense phosphorothioate and 2'-*O*-funtionalized oligonucleotide analogues as therapeutic agents for targeting mRNA in cells and *in vivo*. However it became clear that antisense activity was severely limited by their poor ability to penetrate cells and reach their desired RNA targets. Following successful projects in the 1990s together with the Karn lab on the HIV-1 Tat and Rev proteins and their interactions with the respective HIV RNA targets, we utilized strongly binding 2'-OMe/LNA mixmers (with Wengel's group in Odense) as antisense inhibitors of HIV Tat-dependent *trans*-activation and also developed (with the Lebleu lab in Montpellier) cell-penetrating peptides (CPP) as conjugates for oligonucleotide cell delivery. Particular success came with CPP delivery of charge-neutral PNA oligonucleotides. Moving to the convenient HeLa pLuc705 splice-switching assay of pre-mRNA, we developed a class of new Arg-rich CPPs called Pip. Teaming up in 2007 with the Wood group in Oxford, we applied such CPPs to delivery of similarly charge-neutral PMOs into muscle cells and into a mouse model of Duchenne muscular dystrophy. This exciting and fruitful collaboration has led us also into peptide-PMO conjugates for treatment of other neuromuscular diseases such as spinal muscular atrophy and myotonic dystrophy. The culmination of this work is leading to ongoing plans for commercialization of this platform technology of PMO delivery by conjugated novel CPPs towards clinical trials, leading me to great optimism for the future.